

CLAIMS

1. A method for preparing an array for authenticating whether a plant sample is originated from a known plant, the method comprising the steps of:

- a) extracting DNAs from the known plant;
- b) amplifying variable regions from the extracted DNAs to obtain nucleotide sequences of the variable regions;
- c) designing specific primers according to the nucleotide sequences;
- d) amplifying the variable regions by nested-PCR with the specific primers to obtain DNA fragments; and
- e) dotting the DNA fragments onto a solid support.

2. The method of claim 1, wherein the variable regions include ITSs, ETSS or IGRs.

3. The method of claim 2, wherein the variable regions are ITS1 and ITS2.

4. The method of claim 3, wherein the known plant is *Ilex asprella*, *Ilex latifolia* or *Ilex rotunda*.

5. The method of claim 4, wherein the specific primers comprise nucleotide sequences selected from the group consisting of: SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO:17, SEQ ID NO: 18, SEQ ID NO:19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO:45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO:52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO:55, and SEQ ID NO:56.

6. The method of claim 4, wherein the step b) further comprises: amplifying the ITS1 region using primers IL-ITS1-143 (SEQ ID NO:7) and IL-ITS1-499R (SEQ ID NO:8) to reduce rRNA 18S and 5.8S regions flanking the ITS1 region of the nucleotide sequences.

7. The method of claim 3, wherein the ITS1 region comprises a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2 and SEQ ID NO:3.

8. The method of claim 4, wherein the ITS1 region comprises a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2 and SEQ ID NO:3.

9. The method of claim 5, wherein the ITS1 region comprises a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2 and SEQ ID NO:3.

10. The method of claim 3, wherein the ITS2 region comprises a sequence selected from the group consisting of: SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:6.

11. The method of claim 4, wherein the ITS2 region comprises a sequence selected from the group consisting of: SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:6.

12. The method of claim 5, wherein the ITS2 region comprises a sequence selected from the group consisting of: SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:6.

13. The method of claim 7, wherein the ITS2 region comprises a sequence selected from the group consisting of: SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:6.

14. The method of claim 8, wherein the ITS2 region comprises a sequence selected from the group consisting of: SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:6.

15. The method of claim 9, wherein the ITS2 region comprises a sequence selected from the group consisting of: SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:6.

16. An array for authenticating whether a plant sample is originated from a known plant, which is prepared by the steps of:

- a) extracting DNAs from the known plant;
- b) amplifying variable regions from the extracted DNAs to obtain nucleotide sequences of the variable regions;
- c) designing specific primers according to the nucleotide sequences;

d) amplifying the variable regions by nested-PCR with the specific primers to obtain DNA fragments; and

e) dotting the DNA fragments onto a solid support.

17. The array of claim 16, wherein the variable regions include ITSs, ETSs or IGRs.

18. The array of claim 17, wherein the variable regions are ITS1 and ITS2.

19. The array of claim 18, wherein the known plant is *Ilex asprella*, *Ilex latifolia* or *Ilex rotunda*.

20. The array of claim 19, wherein the specific primers comprise nucleotide sequences selected from the group consisting of: SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, and SEQ ID NO: 56.

21. The array of claim 19, wherein the step b) further comprises:
amplifying the ITS1 region using primers IL-ITS1-143 (SEQ ID NO:7) and IL-ITS1-499R (SEQ ID NO:8) to reduce rRNA 18S and 5.8S regions flanking the ITS1 region of the nucleotide sequences.

22. The array of claim 20, wherein the step b) further comprises:
amplifying the ITS1 region using primers IL-ITS1-143 (SEQ ID NO:7) and IL-ITS1-499R (SEQ ID NO:8) to reduce rRNA 18S and 5.8S regions flanking the ITS1 region of the nucleotide sequences.

23. The array of claim 18, wherein the ITS1 region comprises a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2 and SEQ ID NO:3.

24. The array of claim 19, wherein the ITS1 region comprises a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2 and SEQ ID NO:3.

25. The array of claim 20, wherein the ITS1 region comprises a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2 and SEQ ID NO:3.

26. The array of claim 18, wherein the ITS2 region comprises a sequence selected from the group consisting of: SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:6.

27. The array of claim 19, wherein the ITS2 region comprises a sequence selected from the group consisting of: SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:6.

28. The array of claim 20, wherein the ITS2 region comprises a sequence selected from the group consisting of: SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:6.

29. A method for determining whether a plant sample is originated from a known plant, wherein the method comprises:

- a) extracting first DNAs from the known plant;
- b) amplifying variable regions from the extracted first DNAs to obtain nucleotide sequences of the variable regions;
- c) designing specific primers according to the nucleotide sequences;
- d) amplifying the variable regions by nested-PCR with the specific primers to obtain DNA fragments;
- e) dotting the DNA fragments onto a solid support to obtain an array;
- f) extracting second DNAs and third DNAs from the plant sample and the known plant, respectively;
- g) respectively amplifying the variable regions from the extracted second and third DNAs to produce sample probes and control probes which are derived from the known plant;
- h) hybridizing the sample and control probes with the array, respectively to obtain corresponding hybridization signals; and
- i) processing the hybridization signals to determine whether the plant sample is originated from the known plant.

30. The method of claim 29, wherein the variable regions include ITSs, ETSs or IGRs.

31. The method of claim 30, wherein the variable regions are ITS1 and ITS2.

32. The method of claim 31, wherein the known plant is *Ilex asprella*, *Ilex latifolia* or *Ilex rotunda*.

33. The method of claim 32, wherein the specific primers comprise nucleotide sequences selected from the group consisting of: SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, and SEQ ID NO: 56.

34. The method of claim 31, wherein the steps b) and g) further comprises: amplifying the ITS1 region using primers IL-ITS1-143 (SEQ ID NO: 7) and IL-ITS1-499R (SEQ ID NO: 8) to reduce rRNA 18S and 5.8S regions flanking the ITS1 region of the nucleotide sequences.

35. The method of claim 29, wherein the step i) comprises comparing the hybridization values of the sample probes to those of the control probes to see whether both of them are identical.

36. The method of claim 29, wherein the step i) comprises plotting a graph of the length of the fragments versus corresponding values of the hybridization signals, and linearly regressing the graph.

37. The method of claim 29, wherein the probes are labeled with a detectable moiety.

38. The method of claim 37, wherein the detectable moiety is dioxigenin.

39. The method of claim 32, wherein the control probes comprise a sequence selected from the group comprising of: SEQ IN NO:1, SEQ IN NO:2, SEQ IN NO:3, SEQ IN NO:4, SEQ IN NO:5 and SEQ IN NO:6.

40. The method of claim 31, wherein the ITS1 region comprises a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2 and SEQ ID NO:3.

41. The method of claim 31, wherein the ITS2 region comprises a sequence selected from the group consisting of: SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:6.

42. A kit for authenticating whether a plant sample is originated from a Chinese medicinal plant of *Ilex asprella*, *Ilex latifolia* or *Ilex rotunda*, comprising:

an array prepared by the steps of:

- a) extracting DNAs from the plant;
- b) amplifying ITS1 and ITS2 regions from the extracted DNAs to obtain nucleotide sequences of the ITS1 and ITS2 regions;
- c) designing specific primers according to the nucleotide sequences;
- d) amplifying the ITS1 and ITS2 regions by nested-PCR with the specific primers to obtain DNA fragments; and
- e) dotting the DNA fragments onto a solid support,

primer pairs selected from SEQ ID NO. 7 and SEQ ID NO. 8, and SEQ ID NO. 59 and SEQ ID NO. 60;

a control probe comprising a sequence selected from the group consisting of: SEQ IN NO:1, SEQ IN NO:2, SEQ IN NO:3, SEQ IN NO:4, SEQ IN NO:5 and SEQ IN NO:6; and

a specification providing an indication of the authentication.

43. The kit of claim 42, wherein the specific primers comprise nucleotide sequences selected from the group consisting of: SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO:17, SEQ ID NO: 18, SEQ ID NO:19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID

NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, and SEQ ID NO: 56.

44. The kit of claim 42, wherein the step b) further comprises:

amplifying the ITS1 region using primers IL-ITS1-143 (SEQ ID NO:7) and IL-ITS1-499R (SEQ ID NO:8) to reduce rRNA 18S and 5.8S regions flanking the ITS1 region of the nucleotide sequences.

45. The kit of claim 43, wherein the step b) further comprises:

amplifying the ITS1 region using primers IL-ITS1-143 (SEQ ID NO:7) and IL-ITS1-499R (SEQ ID NO:8) to reduce rRNA 18S and 5.8S regions flanking the ITS1 region of the nucleotide sequences.

46. The kit of claim 42, wherein the ITS1 region comprises a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2 and SEQ ID NO:3.

47. The kit of claim 43, wherein the ITS1 region comprises a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2 and SEQ ID NO:3.

48. The kit of claim 42, wherein the ITS2 region comprises a sequence selected from the group consisting of: SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:6.

49. The kit of claim 43, wherein the ITS2 region comprises a sequence selected from the group consisting of: SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:6.

50. The kit of claim 46, wherein the ITS2 region comprises a sequence selected from the group consisting of: SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:6.

51. The kit of claim 47, wherein the ITS2 region comprises a sequence selected from the group consisting of: SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:6.